

AFW  
A31  
MT

W2  
A2  
F17d

~~30425~~

30425  
~~MEDICAL PREVENTIVE~~

GENERAL HEADQUARTERS  
FAR EAST COMMAND  
MILITARY INTELLIGENCE SECTION, GENERAL STAFF  
ALLIED TRANSLATOR AND INTERPRETER SECTION

"Experimentation with Shigella dysenteriae and Shigella  
para-dysenteria," by Army Medical College Epidemiology Laboratory,  
31 Mar 42.

Doc No 55388C

689253

*Medicine - research  
Dysentery*

NATIONAL  
LIBRARY  
AUG 2 1957  
OF  
MEDICINE



131  
med 2-1

UNCLASSIFIED

COUNTRY Japan

REPORT NO. TR-765-50 WDCS - INTELLIGENCE REPORT I.D. NO. 689253

SUBJECT: "Experimentation with Shigella dysenteriae and Shigella para-dysenteriae"

FROM: T/T, G-2, GHQ, FEC REFERENCES:

EVALUATION: DATE OF INFORMATION: 1942 DATE OF REPORT: 31 July 1950

INCL. ONE PREPARED BY: ATIS, G-2, GHQ, FEC SOURCE: former Japanese Army

SUMMARY OR SID REPORT:

Forwarded herewith four (4) copies of Allied Translator and Interpreter Section Document Number 553880, dated 11 Feb 50. This is a full translation of "Experimentation with Shigella dysenteriae and Shigella para-dysenteriae", a former Japanese Army report by the Army Medical College Epidemiology Laboratory, 31 Mar 42. This report describes experimental procedure and presents conclusions drawn from observations of the cellucidal time, bacterial count and turbidity in the microscopic studies on the destruction of Shigella dysenteriae and Shigella peradysenteriae suspensions which were treated on the same energy level with supersonic waves at frequencies of 1120, 560 and 280 kc.

For the Assistant Chief of Staff, G-2:

1 Inclosure  
4 copies ATIS Doc. No. 553880

J. H. POLK  
Lt Col, GSC  
Executive

NOTE: This document contains information affecting the national defense of the United States within the meaning of the Espionage Act, 50 U.S.C. 31 and 32, as amended. Its transmission or the revelation of its contents in any manner to an unauthorized person is prohibited by law.

UNCLASSIFIED

MANUFACTURED BY OLD TOWN RIDGON & CARSON COMPANY, BROOKLYN, N.Y. APRIL 5, 1949

131  
med 2-1

UNCLASSIFIED

COUNTRY Japan

REPORT NO. TR-795-50 WDCS - INTELLIGENCE REPORT I.D. NO. 693458

SUBJECT: Experimentations with Cholera Bacteria

FROM: T/T, G-2, GHQ, FEC REFERENCES:

EVALUATION: DATE OF INFORMATION: 1935-1942 DATE OF REPORT: 11 Aug 1950

INCL. ONE PREPARED BY: ATIS, G-2, GHQ, FEC SOURCE: Former Japanese Army Medical College

SUMMARY OR SID REPORT:

Forwarded herewith four (4) copies each of the following Allied Translator and Interpreter Section documents (bound in one cover):

- Doc No. 55388-A "Supersonic Wave Generator; Experimentation with Cholera," by Army Medical College Epidemiology Laboratory, 31 March 1942.
- Doc. No. 55388-D "Serological Studies on Supersonic Wave-Treated Polyvalent Cholera Vaccine," by Army Medical College, Epidemiological Laboratory, 16 June 1942. (full translation)
- Doc. No. 55388-E "Experimental Research on Toxic Fractions of U.S.W. Cholera Vaccine," by Epidemiological Laboratory, Army Medical College, 1940 (full translation)
- Doc. No. 55388-F "Research on Cholera Vaccines Treated with Supersonic Waves; Effects of Formalin on Antigenic Properties," by Epidemiological Laboratory of Army Medical College, 1939 (full translation)

NOTE: This document contains information affecting the national defense of the United States within the meaning of the Espionage Act, 50 U.S.C. 31 and 32, as amended. Its transmission or the revelation of its contents in any manner to an unauthorized person is prohibited by law.

UNCLASSIFIED

MANUFACTURED BY OLD TOWN RIDGON & CARSON COMPANY, BROOKLYN, N.Y. APRIL 5, 1949



U.S. Army GENERAL HEADQUARTERS  
FAR EAST COMMAND  
(MILITARY INTELLIGENCE SECTION, GENERAL STAFF)  
ALLIED TRANSLATOR AND INTERPRETER SECTION

Translation Requested by Theatre Intell, Targets

Date Rec'd AFIS 11 Feb 50

Description of Contents: Full translation of "Experimentation with Shigella dysenteriae and Shigella paradysenteriae," by Army Medical College Epidemiology Laboratory, 31 Mar 42.

GENERAL HEADQUARTERS  
THE EAST COMMAND

U.S. Army

MILITARY INTELLIGENCE SECTION, GENERAL STAFF  
ALLIED TRANSLATION AND INTERPRETING SECTION

Translation requested by Chinese Intelligence, Tokyo

Date Rec'd ATIS 11 Feb 50

Description of Contents: Full translation of "Report on the  
epidemic of dysentery and Shigella par-  
dysenteriae," by Army Medical College  
Epidemiology Laboratory, 11 Mar 49.



G12F57

Army Medical College Epidemiological Research Report

Section 2. Number 331

Relationship of Supersonic Wave  
Frequency and Celluloidal Action

Part 3. Experimentation with Shigella  
Dysenteriae and Shigella paradysenteriae.

Army Medical College  
Epidemiology Laboratory  
(Maj Gen ISHII,  
Commanding)  
ENDO, Takeshi  
Non-official staff

Section 2
Original Copy
Classification
376
376-33
433-1
Received 31 Mar 42

## Table of Contents

### General

Chapter I. Outline of experiment.

Chapter II. Experimental procedure.

- A. Bacterial strain.
- B. Bacterial suspension.
- C. Supersonic wave treatment.
- D. Qualitative tests on cellulicidal strength.
- E. Bacterial count.
- F. Observation of morphological changes.
- G. Measurement of turbidity.

Chapter III. Results of experiment.

- A. Cellulicidal time.
- B. Survival test.
- C. Observation of morphological changes.
- D. Measurement of turbidity.

Chapter IV. Summary and conclusions.

Bibliography



## General

In recent times studies on the detoxication of dysentery vaccines have been performed in various quarters. Again, the separation of endotoxins upon destroying the dysentery bacillus cells with supersonic waves has already been reported by several persons. FUNATO has claimed that at a frequency of 560 kc the *Shigella dysenteriae* can be destroyed in 20 minutes, the *Shigella paradysenteriae* I in 10 minutes and the *Shigella paradysenteriae* III in 30 minutes. CEAXI has studied the relationship of cellulicide and turbidity to temperatures.

Nevertheless, reports on cellulicide tests employing different frequencies but conducted on the same energy level have not been published as yet. The varying of the destructive effect directly received by the bacterial cell through frequency changes is not believed to be difficult. Following this line of reasoning experiments were performed on the destruction of *Shigella dysenteriae* and *Shigella paradysenteriae* at different bacterial concentrations and frequencies.

## Chapter I. Outline of experiment.

The treatment of bacterial suspensions as well as the examination of surviving cells, bacterial count, morphology and turbidity was based on procedures outlined in Part I (cholera bacteria) of this series. Experiments were performed on *Shigella dysenteriae* (Sugimori strain) and *Shigella paradysenteriae* (F type).

## Chapter II. Experimental procedure.

A. Bacterial strain: The bacteria were from strains kept at this laboratory. These strains indicated uniform turbidity in a bouillon medium and gas was absent in lactose and dextrose vertical media. Milk coagulation was negative. Litmus and whey tests were negative. They were negative in lead acetate agar media. Indol reaction was negative for *Shigella dysenteriae* but positive for the F type. *Shigella dysenteriae* was negative in the decomposition of carbohydrates such as xylose, rhamnose, sorbit, maltose, arabinose and saccharose. *Shigella paradysenteriae* was able to decompose maltose and arabinose but not the others. Agglutination titer was 800 times for *Shigella dysenteriae* (immune sera prepared by Immunological Section) and 1,280 times for *Shigella paradysenteriae* (prepared by this laboratory).

B. Bacterial suspension: Bacterial suspensions were prepared in the same manner as that for typhoid bacilli in Part 2 of this series.

C. Supersonic wave treatment: As already described in Part I, the plate current was regulated to produce a 10° C-per-minute temperature rise in a test tube containing a specific volume of transformer oil for the purpose of maintaining a constant supersonic wave energy level in the test tube at each frequency. The following currents were used to provide the same energy level:

<u>Frequency</u>	<u>Plate current</u>	<u>Grid current</u>	<u>Plate voltage</u>
1120 kc	460 ma	165 ma	3000 v
560 kc	550 ma	130 ma	3300 v
280 kc	400 ma	190 ma	3300 v



Again following the same procedure as in Part 1 the treatment time at 1120, 560 and 280 kc was 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135 and 150 minutes.

D. Qualitative tests on cellulicidal strength: Two platinum loop portions each of the supersonic wave-treated bacterial suspensions were slant-cultured and plate-cultured with agar and bouillon. After incubating at 37° C for 24 hours the results were evaluated as ++, ++, + and - according to their growth.

E. Bacterial count: The procedure described in Part 2 was followed. The supersonic wave-treated bacterial solutions were diluted progressively ten times each with a physiological saline solution. One cc of each was mixed thoroughly with agar (45° C) and allowed to solidify. Agar was added again in an amount sufficient to cover the surface. Bacterial counts were taken after incubating at 37° C for 24 hours (48 hours in case of unsatisfactory growth). The counting method has already been described in Part 1.

As the purpose of this experiment was to determine the number of destroyed bacteria, bacterial counts were taken on the control and also during the intermediate stage and the stage immediately prior to destruction. The rate of time decrease compared to the bacterial count of the control was examined.

F. Observation of morphological changes: Smear specimens were prepared immediately following the supersonic wave treatment. Microscopic observations were made after the specimens were dried, fixed and single-stained with methylene blue.

G. Measurement of turbidity: The bacterial suspensions were diluted with a physiological saline solution according to the ratios shown below and measured with a Pulfrich's photometer. The absolute turbidity was computed from the relative turbidity.

<u>Bacterial solution concentration</u>	<u>Dilution</u>
0.1 mg / 1.0 cc	Stock solution
1.0 mg / 1.0 cc	10 times
10.0 mg / 1.0 cc	100 times

Chapter III. Results of experiment. (See Tables 1-6.)



Table 1. Test results on cellulicidal strength; survival and turbidity of *Shigella dysenteriae* treated with 1120 kc supersonic waves.

Plate voltage 3000 v; plate current 460 ma; grid current 185 ma. Date of experiment 14 Apr 41. Room temperature 29° C.

0.1 mg						1.0 mg						10.0 mg								
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (Z)	Survival compared to con- trol (%)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (Z)	Survival compared to con- trol (%)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (Z)	Survival compared to con- trol (%)	Log Z
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
K	++	++	0.013	58.7 x 10 <sup>6</sup>		7.7686	K	+++	+++	0.1037	58.7 x 10 <sup>6</sup>		8.7686	K	++	+++	0.8923	5870 x 10 <sup>6</sup>		9.7686
1	+	+	0.012				1	+++	+++	0.0981				1	++	+++	0.8355			
2	+	+	0.012				2	++	++	0.0738				2	++	+++	0.7919			
3	+	+	0.012	0.000188 x 10 <sup>6</sup>	0.000269	2.1937	3	++	++	0.0691	13.5 x 10 <sup>6</sup>	5.3862	7.4983	3	++	++	0.6131			
4	—	—	0.012				4	++	++	0.0680				4	++	++	0.5577			
5	—	—	0.011				5	++	++	0.0680	3.56 x 10 <sup>6</sup>	0.6064	6.5514	5	++	++	0.5353			
6	—	—	0.012				6	++	++	0.0663				6	++	++	0.5242			
7	—	—	0.010				7	—	—	0.0725				7	++	++	0.4284			
8	—	—	0.012				8	—	—	0.0791				8	++	++	0.4907			
9	—	—	0.014				9	—	—	0.0803				9	++	++	0.5019			
10	—	—	0.014				10	—	—	0.0981				10	++	++	0.5186			
15	—	—	0.014				15	—	—	0.1138				20	+	+	0.5298	212.6 x 10 <sup>6</sup>	0.3611	8.3275
							20	—	—	0.1360				40	+	+	0.6134	7.69 x 10 <sup>6</sup>	0.1310	6.8859
							30	—	—	0.1360				50	+	+	0.5855	0.45 x 10 <sup>6</sup>	0.000076	5.6532
														60	—	—	0.9644			
														75	—	—	0.8750			



Table 2. Test results on cellulicidal strength; survival and turbidity of *Shigella dysenteriae* treated with 560 kc supersonic waves.

Plate voltage 3000 v; plate current 550 ma; grid current 130 ma. Date of experiment 11 Jun 41. Room temperature 26° C.

0.1 mg						1.0 mg						10.0 mg								
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (Z)	Survival compared to con- trol (%)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (Z)	Survival compared to con- trol (%)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (Z)	Survival compared to con- trol (%)	Log Z
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
K	++	++	0.00714	$54.91 \times 10^6$		7.7775	K	+++	+++	0.08081	$599.1 \times 10^6$		3.7775	K	+++	+++	0.32540	$3991.650 \times 10^6$		9.775
1	++	++	0.01528				1	+++	+++	0.07808				1	+++	+++	0.62462			
2	++	++	0.01717				2	+++	+++	0.07473				2	+++	+++	0.59000			
3	++	++	0.01168	$0.259 \times 10^6$	0.432	5.4133	3	+++	+++	0.06246	$8.143 \times 10^6$	1.525	6.9108	3	+++	+++	0.63578			
4	++	++	0.01253				4	+++	+++	0.05967				4	+++	+++	0.63020			
5	+	+	0.01199	$0.031 \times 10^6$	0.052	4.4771	5	+++	+++	0.06157	$2.820 \times 10^6$	0.470	6.4502	5	+++	+++	0.596886			
6	+	+	0.01127				6	+++	+++	0.06581				6	+++	+++	0.63578			
7	+	+	0.01048				7	+++	+++	0.06912				7	+++	+++	0.53539			
8	-	-	0.01026				8	++	++	0.06577	$1.968 \times 10^6$	0.329	6.2941	8	++	++	0.55770			
9	-	-	0.01004				9	++	++	0.06465				9	++	++	0.51308			
10	-	-	0.00982				10	+	+	0.06967	$0.119 \times 10^6$	0.020	5.0755	10	++	++	0.52424			
15	-	-	0.00948				15	+	+	0.09246	$0.108 \times 10^6$	0.019	5.0334	20	+	+	0.41828	$4.750 \times 10^6$	0.071	6.6772
20	-	-	0.00982				20	+	+	0.07696	$0.099 \times 10^6$	0.016	4.9956	30	+	+	0.48520	$1.976 \times 10^6$	0.003	6.2958
30	-	-	0.01312				30	+	+	0.08087	$0.070 \times 10^6$	0.012	4.8451	40	+	+	0.50193	$0.905 \times 10^6$	0.0015	5.9566
40	-	-	0.91239				40	+	+	0.08142	$0.007 \times 10^6$	0.001	3.8451	50	+	+	0.45731	$0.667 \times 10^6$	0.009	5.8241
							50	-	-	0.07585				60	+	+	0.42943	$0.559 \times 10^6$	0.009	5.8814
							60	-	-	0.09927				75	+	+	0.50183	$0.306 \times 10^6$	0.005	5.4857
							75	-	-	0.1039				90	+	+	0.41270			
														105	+	+	0.51867	$0.297 \times 10^6$	0.004	5.4728
														120	+	+	0.53540	$0.226 \times 10^6$	0.003	5.3541
														135	+	+	0.55213	$0.0186 \times 10^6$	0.00003	4.2696
														150	-	-	0.66586			



Table 5. Test results on cellulicidal strength, survival and turbidity of *Shigella dysenteriae* treated with 280 kc supermic waves.

Plate voltage 3500 v; plate current 400 ma; grid current 190 ma. Date of experiment 10 Jul 41. Room temperature 28°C.

0.1 mg							1.0 mg							10.0 mg						
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg ( $\bar{x}$ )	Survival compared to con- trol (%)	Log $\bar{x}$	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg ( $\bar{x}$ )	Survival compared to con- trol (%)	Log $\bar{x}$	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg ( $\bar{x}$ )	Survival compared to con- trol (%)	Log $\bar{x}$
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
K	+++	+++	0.00872	$57.879 \times 10^6$		7.7609	K	+++	+++	0.00971	$576.790 \times 10^6$		8.7609	K	+++	+++	0.13255	$5767.900 \times 10^6$		8.7609
1	+++	+++	0.00881	$15.866 \times 10^6$	25.128	7.1561	1	+++	+++	0.05242				1	+++	+++	0.57443			
2	+++	+++	0.00878				2	+++	+++	0.04974				2	+++	+++	0.51539			
3	+++	+++	0.00880	$2.715 \times 10^6$	4.708	6.4338	3	+++	+++	0.05400	$47.426 \times 10^6$	25.363	8.7725	3	+++	+++	0.40077			
4	+++	+++	0.00869				4	+++	+++	0.04963				4	+++	+++	0.46329			
5	+++	+++	0.00882	$0.076 \times 10^6$	0.119	4.8976	5	+++	+++	0.04985				5	+++	+++	0.43425			
6	++	++	0.00882				6	++	++	0.05331				6	+++	+++	0.47982			
7	++	++	0.00880				7	++	++	0.05577				7	+++	+++	0.47962			
8	++	++	0.00818	$0.004 \times 10^6$	0.007	5.6021	8	++	++	0.06124	$2.125 \times 10^6$	0.364	6.3229	8	+++	+++	0.61342			
9	-	-	0.00882				9	+	+	0.06135				9	+++	+++	0.59116			
10	-	-	0.00708				10	+	+	0.05987	$1.628 \times 10^6$	0.294	6.2116	10	++	+++	0.49635			
							15	+	+	0.04796	$0.826 \times 10^6$	0.091	6.7160	15	++	+++	0.39697	$561.612 \times 10^6$	0.627	8.5588
							20	-	-	0.05577				20	+	++	0.39617	$0.972 \times 10^6$	0.0171	5.9877
							25	-	-	0.06892				25	-	-	0.37623			
														30	-	-	0.41271			
														40	-	-	0.33808			



Table 4. Test results on cellulicidal strength; survival and turbidity of *Shigella paradyenteriae* treated with 1120 kc ultrasonic waves.  
Plate voltage 3000 v; plate current 400 ma; grid current 105 ma. Date of experiment 19 Apr 41. Room temperature 29° C.

0.1 mg							1.0 mg							10.0 mg						
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (x)	Survival compared to control (x)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (x)	Survival compared to control (x)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (x)	Survival compared to control (x)	Log Z
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
K	+++	+++	0.01085	50.35 x 10 <sup>5</sup>		7.4847	K	+++	+++	0.00369	375.3 x 10 <sup>5</sup>		8.4847	K	+++	+++	1.142	3083 x 10 <sup>5</sup>		9.4847
1	+++	+++	0.01360				1	+++	+++	0.00250				1	+++	+++	0.691			
2	++	++	0.01026				2	++	++	0.00098				2	+++	+++	0.631			
3	++	++	0.00936	0.0064 x 10 <sup>5</sup>	0.0209	8.8062	3	++	++	0.07584				3	+++	+++	0.959			
4	+	+	0.00925				4	++	++	0.06092				4	+++	+++	0.736			
5	+	+	0.00892	0.0063 x 10 <sup>5</sup>	0.00206	3.7993	5	++	++	0.00580				5	+++	+++	0.728			
6	+	+	0.00836				6	+	+	0.06023				6	+++	+++	0.790			
7	+	+	0.0049				7	+	+	0.05200				7	+++	+++	0.691			
8	—	—	0.01003				8	+	+	0.05577	26.6 x 10 <sup>5</sup>	8.7127	7.4249	8	++	++	0.659			
9	—	—	0.0245				9	—	—	0.02134				9	++	++	0.655			
10	—	—	0.01716				10	+	+	0.00409	8.921 x 10 <sup>5</sup>	2.999	6.9435	10	+	+	0.713	277 x 10 <sup>5</sup>	90.0673	8.4425
							15	+	+	0.05877	0.67 x 10 <sup>5</sup>	0.2190	5.9201	15	+	+	0.438	297 x 10 <sup>5</sup>	67.8000	8.3160
							20	—	—	0.01212				20	—	—	0.702	0.015 x 10 <sup>5</sup>	0.0049	4.1761
														30	+	+	0.702	0.0058 x 10 <sup>5</sup>	0.0018	3.7054
														35	+	+				
														40	+	+	0.719			
														45	—	—	0.948			



Table 5. Test results on celluloidal strength, survival and turbidity of *Shigella paradysenteriae* treated with 500 kc superonic waves.

Plate voltage 5000 v; plate current 550 ma; grid current 150 ma. Date of experiment 8 Jan 41. Room temperature 25°C.

0.1 mg							1.0 mg							10.0 mg						
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (2)	Survival compared to control (4)	Log 2	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (2)	Survival compared to control (5)	Log 2	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (2)	Survival compared to control (5)	Log 2
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
0	+++	+++	0.00093	$35.903 \times 10^6$		7.5558	0	+++	+++	0.07194	$350.030 \times 10^6$		8.8588	0	+++	+++	1.08782	$2896.300 \times 10^6$		9.558
1	+++	+++	0.0274	$0.511 \times 10^6$	1.421	6.7189	1	+++	+++	0.08204				1	+++	+++	0.00238			
2	+++	+++	0.04131				2	+++	+++	0.07829				2	+++	+++	0.02580			
3	+++	+++	0.01248	$0.498 \times 10^6$	1.325	6.0772	3	+++	+++	0.07027				3	+++	+++	0.01424			
4	+++	+++	0.01292				4	+++	+++	0.06204				4	+++	+++	0.07133			
5	+++	++	0.01469	$0.190 \times 10^6$	0.523	5.2793	5	++	++	0.06204				5	+++	+++	0.02501			
6	+++	++	0.01277				6	++	++	0.07027				6	+++	+++	0.04770			
7	+++	++	0.01902				7	++	++	0.07250				7	+++	+++	0.05579			
8	++	++	0.02187	$0.072 \times 10^6$	0.200	4.8573	8	++	++	0.07027	$350.003 \times 10^6$	74.900	8.5438	8	+++	+++	0.02270			
9	++	++	0.01593				9	++	++	0.06460				9	+++	+++	0.09116			
10	++	++	0.01513				10	++	++	0.06492				10	+++	+++	0.04893			
15	+	+	0.01149				15	++	++	0.05577	$300.227 \times 10^6$	66.100	8.4011	15	+++	+++	0.04616			
20	+	+	0.01174				20	++	++	0.00023				20	+++	+++	0.03402			
30	+	+	0.01202	$0.088 \times 10^6$	0.180	4.8129	30	+	+	0.05263	$300.952 \times 10^6$	60.027	8.4023	30	+++	+++	0.07327	$353.194 \times 10^6$	0.007	8.5628
40	-	-	0.01223				40	+	+	0.04064	$452.3 \times 10^6$	1.252	6.0256	40	+++	+++	0.02338	$122.690 \times 10^6$	0.369	8.0985
50	-	-	0.02187				50	+	+	0.05405				50	+++	+++	0.03025	$41.424 \times 10^6$	0.111	7.6162
							75	-	-	0.05200				75	++	++	0.12731	$7.014 \times 10^6$	0.020	6.8459
							90	-	-	0.05323				90	+	+	0.13943	$0.388 \times 10^6$	0.001	5.5908
							105	-	-	0.15202				105	+	+	0.11154	$0.303 \times 10^6$	0.0008	5.4814
							120							120	+	+	0.11731	$0.197 \times 10^6$	0.0003	5.0904
							135							135	+	+	0.13058	$0.088 \times 10^6$	0.0001	4.9106
							150							150	-	-	0.12269			



Table 6. Test results on cellulicidal strength; survival and turbidity of *Shigella paradysoenteriae* treated with 220 kc supermic waves.

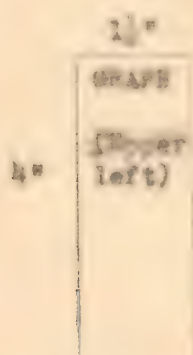
Plate voltage 3300 v; plate current 400 ma; grid current 100 ma. Date of experiment 9 Jul 41. Room temperature 32°C.

0.1 mc						1.0 mc						10.0 mc								
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mc (x)	Survival compared to con- trol (%)	Log 2	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mc (x)	Survival compared to con- trol (%)	Log 2	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mc (x)	Survival compared to con- trol (%)	Log 2
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
0	++	++	0.00959	$50.652 \times 10^3$		7.4294	0	++	++	0.00700	$306.590 \times 10^3$		0.4094	0	++	++	0.00208	$3005.0 \times 10^3$		0.4094
1	++	++	0.02348				1	++	++	0.02561				1	++	++	0.01434			
2	++	++	0.01616				2	++	++	0.02911				2	++	++	0.02208			
3	++	++	0.00731				3	++	++	0.05577				3	++	++	0.02402			
4	++	++	0.00591				4	++	++	0.05384				4	++	++	0.00133			
5	++	++	0.00746				5	++	++	0.04796	$3.010 \times 10^3$	1.100	0.5588	5	++	++	0.01270			
6	++	++	0.00705				6	++	++	0.04517				6	++	++	0.03462			
7	—	—	0.00519				7	++	++	0.04461				7	++	++	0.05137			
8	—	—	0.00714				8	++	++	0.04573	$1.606 \times 10^3$	0.858	0.2205	8	++	++	0.03113			
9	—	—	0.00705				9	++	++	0.04624				9	++	++	0.04577			
10	—	—	0.00656				10	++	++	0.04352	$1.583 \times 10^3$	0.516	0.1405	10	++	++	0.07625			
15	—	—	0.00624				15	++	++	0.04461	$0.002 \times 10^3$	0.0006	0.3010	15	++	++	0.04859	$21.242 \times 10^3$	0.494	7.3275
							20	—	—	0.05577				20	+	+	0.04702	$2.489 \times 10^3$	0.021	0.6989
							25	—	—	0.04461				25	+	+	0.12354	$0.905 \times 10^3$	0.029	0.9586
							30	—	—	0.03513				30	—	—	0.12662			
														40	—	—	0.17646			
														50	—	—	0.12781			
														60	—	—	0.17289			



The survival times of bacteria are listed in Figures 1 to 6; morphological changes are shown in Tables 7 and 8.

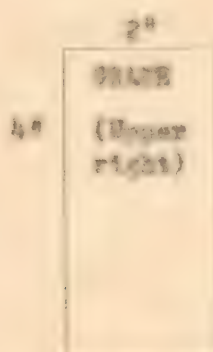
Figure 1. Bacterial count variations of 0.1 mg/  
1.0 cc bacterial (*Shigella dysenteriae*) solution



Key

- (1) Bacterial count.
- (2) (Stock solution).
- (3) Time (minutes).

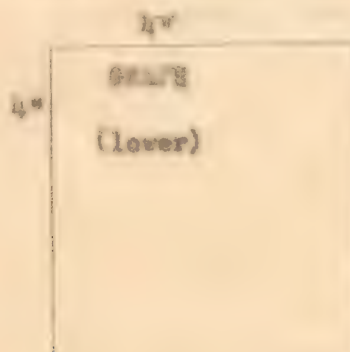
Figure 2. Bacterial count variations of 1.0 mg/  
1.0 cc bacterial (*Shigella dysenteriae*) solution



Key

- (1) Bacterial count.
- (2) Time (minutes).

Figure 3. Bacterial count variations of 10.0 mg/  
1.0 cc bacterial (*Shigella dysenteriae*) solution

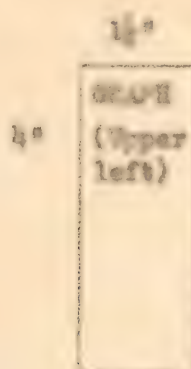


Key

- (1) Bacterial count.
- (2) (Stock solution).
- (3) Time (minutes).



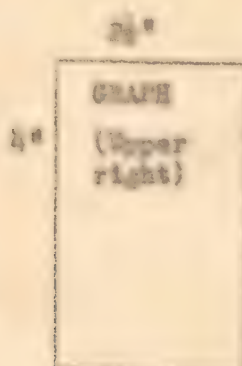
Figure 4. Bacterial count variations of 0.1 mg/  
1.0 cc bacterial (*Shigella paradysenteriae*) solution



Key

- (1) Bacterial count.
- (2) (Stock solution).
- (3) Time (minutes).

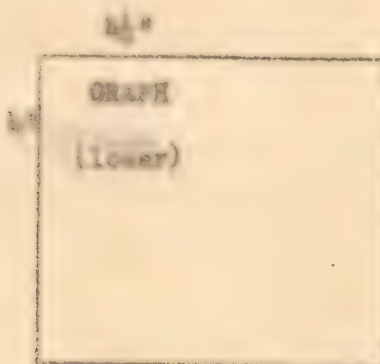
Figure 5. Bacterial count variations of 1.0 mg/  
1.0 cc bacterial (*Shigella paradysenteriae*) solution



Key

- (1) Bacterial count.
- (2) Time (minutes).

Figure 6. Bacterial count variations of 10.0 mg/  
1.0 cc bacterial (*Shigella paradysenteriae*) solution



Key

- (1) Bacterial count.
- (2) (Stock solution).
- (3) Time (minutes).



## A. Cellulicidal time.

Bacterial concentration		0.1 mg	1.0 mg	10.0 mg
Bacterial type	Frequency (kc)			
Shigella dysenteriae	1120	3	7	60
	560	8	50	150
	280	9	20	25
Shigella paradysenteriae	1120	8	20	75
	560	40	60	150
	280	7	20	30

At the same supersonic wave energy level the cellulicidal time for both bacterial types is accelerated at higher dilutions and lower frequencies. (At 1120 kc acceleration was noted in the 0.1 mg and 1.0 mg concentrations.)

The values obtained for the *Shigella dysenteriae* at 1120 kc are three minutes for 0.1 mg and seven minutes for 1.0 mg. At 280 kc cellulicide occurs in 25 minutes for the 1.0 mg concentration. Destruction of *Shigella paradysenteriae* at 280 kc occurs in seven minutes for 0.1 mg, 20 minutes for 1.0 mg and 30 minutes for 10.0 mg. Cellulicidal strength is the highest at 1120 kc followed by 280 kc; this action is retarded at 560 kc. As cases arise where this strength is inversely proportionate to the wave length, some amount of fluctuation is produced.

B. Survival test: (See Figures 1 to 6.) The graphs show a logarithmic decrease of bacterial count after a supersonic wave treatment. By taking the bacterial counts in the graphs as logarithmic scales (vertical axes) and the treatment times as arithmetical scales (horizontal axes), approximately straight lines are formed. A step-like decrease is indicated by the 10.0 mg concentration. By treating the bacterial count as  $y$  and the treatment time as  $x$ , the relationship

$$\begin{aligned}\log y &= a, \\ \text{or } y &= b \times x \\ (a &= \text{negative constant})\end{aligned}$$

is obtained.

Though not distinct in Figures 1, 2, and 5, a sharp reduction in live bacterium count can be seen by examining the bacterial counts for the 10.0 mg/1.0 cc concentration shown in Figures 3 and 6. At the same energy level this reduction becomes pronounced as the frequency decreases or, in other words, as the wave length increases.

The time required for the log of the live bacterium count of the 10.0 mg/1.0 cc concentration to become halved is shown below.

Shigella dysenteriae	1120 kc	Approximately 60 min.
	560 kc	" 40 min.
	280 kc	" 20 min.
Shigella paradysenteriae	1120 kc	Approximately 45 min.
	560 kc	" 30 min.
	280 kc	" 25 min.



C. Observation of morphological changes: A condensation of the results from Tables 7 and 8 is presented below.



Frequency (hr)		1120			560			280		
Bacterial weight (mg)		0.1	1.0	10.0	0.1	1.0	10.0	0.1	1.0	10.0
Shigella dysenteriae	1. Normal bacterial type (cellulicidal time)	3	6	50	7	10	135	9	20	20
	2. Inability to stain	-	-	5	2	5	9	2	2	5
	3. Cellular swelling	-	-	7	2	1	5	2	2	1
	4. Cellular deformation	-	-	10	-	10	5	2	2	2
	5. Cellular destruction	1	2	4	2	1	20	2	2	1
	6. Reminiscence of protoplasts	-	-	10	2	8	5	2	2	2
	7. Destroyed cellular matter - granular cloudy dust-like	-	-	30	-	10	10	-	3	6
		-	-	50	-	90	8	-	-	4
		3	2	7	2	1	2	2	2	1
Shigella paradysenteriae	1. Normal bacterial type (cellulicidal time)	8	15	50	30	50	135	6	15	25
	2. Inability to stain	4	6	8	-	4	3	6	-	6
	3. Cellular swelling	3	3	8	1	2	7	-	2	8
	4. Cellular deformation	-	3	2	-	-	9	-	7	8
	5. Cellular destruction	2	3	7	-	2	3	-	2	4
	6. Reminiscence of protoplasts	4	7	7	-	-	6	-	2	4
	7. Destroyed cellular matter - granular cloudy dust-like	-	-	15	-	60	15	2	2	1
		-	-	20	30	9	15	3	-	2
		2	4	8	8	4	2	-	5	2

Note: Cellulicidal time (cellular destruction) is listed after "Normal bacterial type." Others show time elapsed since treatment began.



D. Measurement of turbidity: As shown in Tables 1 to 6, transparency was not observed among suspensions of *Shigella dysenteriae* and *Shigella paradysenteriae* within the supersonic wave frequency range covered by this experiment. A change was detected only in the 10.0 mg concentration of *Shigella paradysenteriae* but changes in the others seemed to be non-existent.

#### Chapter IV. Summary and conclusions.

The following conclusions were reached after observing the cellulicidal time, bacterial count and turbidity and after making microscopic studies on the destruction of *Shigella dysenteriae* and *Shigella paradysenteriae* suspensions which were treated on the same energy level with supersonic waves at frequencies of 1120, 950 and 280 kc.

A. At the same supersonic wave energy level the cellulicidal time for concentrations up to and including 1.0 mg is accelerated at 1120, 280 and 950 kc, in that order. The time for 10.0 mg is accelerated only at 280 kc. Consequently, the time at which cellulicide occurs is directly proportionate to the frequency and somewhat inversely proportionate to the wave length. A slightly greater amount of difficulty is experienced in the destruction of *Shigella paradysenteriae* compared to *Shigella dysenteriae*.

B. The cellulicidal time decreased as the bacterial concentration is lessened.

C. The changes produced in live bacteria, when based on treatment time, can be expressed logarithmically and possess roughly the following relationship:

$$\log y = ax$$

( $a$  = negative constant).

D. A microscopic examination of the specimens reveals cellular destruction occurring at 280 kc, 1120 kc and 950 kc, in that order.

E. Transparency does not result when *Shigella dysenteriae* and *Shigella paradysenteriae* diluted with a physiological saline solution are treated with supersonic waves. A slight change can be noticed in the case of the 10.0 mg/1.0 cc concentration but changes in the others are practically unobserved.

F. Cellular destruction can not be gauged by changes in turbidity.

#### Bibliography

1. FUKUDA, Jun, Destructive Action on Bacteria with Supersonic Waves, Second Report - Experiments with *Bacillus coli*, *Bacillus typhi*, *Bacillus paratyphi* and *Bacillus dysenteriae*, NIPPON NISSHIMBU HYGIENIC JASSHI (Japan Journal of Microbiology and Pathology), Vol. 32, No. 6, June 1938.

2. UZAKI, Shigeki, Relation between Internal Temperatures and Cellulicidal Action, Turbidity Changes and Volumetric Changes of Bact. Suspensions Treated with Supersonic Waves, NIPPON NISSHIMBU HYGIENIC JASSHI, Vol. 32, No. 10, October 1938.



3. OIASI, Shigeki, Volumetric and Turbidity Changes Occurring in Bacterial Solutions Treated over Extensive Periods with Supersonic Waves, NIPPON SIKIMANVET EYUJIGAKU ZASSHI, Vol. 37, No. 10, October 1938.

4. MURANO, Kiyo, Effects of Supersonic Waves on Antibodies (Second Report) - Experimentation with Complement Fixation Substances, NIPPON SIKIMANVET EYUJIGAKU ZASSHI, Vol. 33, No. 3, March 1939.

5. MURANO, Kiyo, Effects of Supersonic Waves on Antibodies (Third Report) - Experimentation with Precipitin, NIPPON SIKIMANVET EYUJIGAKU ZASSHI, Vol. 33, No. 5, May 1939.



Table 7. Microscopic observations of morphological changes in Shigella dysenteriae at various supersonic wave																														
Frequency		1120 kc										560 kc					280 kc													
		Microscopic observations	Cellular swelling	Morpho-logical deforma-tion	Cellular destruc-tion	Protoplasma (homo-genization)	Granular	Cloudy	Dust-like	Normal	Inability to stain	Cellular swelling	Morpho-logical deforma-tion	Cellular destruc-tion	Proto-plasma (homo-genization)	Granular	Cloudy	Dust-like	Normal	Inability to stain	Cellular swelling	Morpho-logical deforma-tion	Cellular destruc-tion	Proto-plasma (homo-genization)	Granular	Cloudy	Dust-like	Normal	Inability to stain	
0.1 mg	Time																													
	K			++					##																					
	1			++					##																					
	2			++					##																					
	3			++					##																					
	4			++					##																					
	5			++					##																					
	6			++					##																					
	7			++					##																					
	8			++					##																					
	9			++					##																					
	10			++					##																					
	15			++					##																					
	20			++					##																					
	30			++					##																					
1.0 mg	K								##																					
	1								##																					
	2								##																					
	3								##																					
	4								##																					
	5								##																					
	6								##																					
	7								##																					
	8								##																					
	9								##																					
	10								##																					
	15								##																					
	20								##																					
	30								##																					
	40								##																					
50								##																						
60								##																						
75								##																						
90								##																						
10.0 mg	K								##																					
	1								##																					
	2								##																					
	3								##																					
	4								##																					
	5								##																					
	6								##																					
	7								##																					
	8								##																					
	9								##																					
	10								##																					
	20								##																					
	30								##																					
	40								##																					
	50								##																					
60								##																						
75								##																						
90								##																						
105								##																						
120								##																						
135								##																						
150								##																						



17







